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Non-coding RNAs: novel players in chromatin-regulation during viral latency

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Chromatin structure plays an essential role during gene expression regulation not only in the case of the host cellular genome, but also during the viral life cycle. Epigenetic chromatin marks thereby define, whether a gene promoter is accessible for the transcription machinery or whether a repressive heterochromatin state is established. The heterochromatin-mediated repression of lytic viral genes results in viral latency, enabling the virus to persist dormant without being recognized by the host immune system, but keeping the potential for reactivation. Arising new systems biology approaches are starting to uncover an unexpected multiplicity and variety of non-coding (nc)RNAs playing important roles during chromatin structure control, likely constituting a novel layer in epigenetic regulation. In this review we give an overview of chromatin-regulatory viral and host cellular ncRNAs and their links to viral latency.

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Introduction

The shift from lytic to latent infection represents an important viral mechanism to escape the host immune system. Viral latency is associated with the transcriptional repression of genes involved in lytic infection, resulting in a dormant viral genome, which remains competent for reactivation. Often this dormant state is maintained by the expression of few latency-associated viral gene products [1,2].

During the last decades, chromatin structural dynamics have gained increasing attention during the establishment of both episomal [3] and proviral latency [4,5]. Upon initial infection, viral DNA — either episomal DNA or integrated proviral DNA — is packed by host cellular chromatin. Epigenetic modifications, such as histone acetylation, histone methylation or DNA methylation alter this chromatin structure and thus determine the availability of gene promoters for the transcription machinery [6]. The chromatin state leading to viral latency is believed to depend on host cellular factors and, in the case of retroviruses, on the site of integration [7].

Recently, non-coding RNAs such as micro (mi)RNAs [8,9], long non-coding (lnc)RNAs [10–12] and natural antisense transcripts (NATs) [13,14] have emerged as being significantly involved in the epigenetic regulation of chromatin structure. Here we introduce the most important chromatin-modifying complexes involved in viral latency and give an overview of host cellular and viral ncRNAs impacting their activity, thus likely being important factors during latency establishment.

Chromatin dynamics associated with viral latency

Numerous mechanisms have been proposed to be involved in establishing and maintaining viral latency. These mechanisms depend on the virus type, the chromatin environment, transcriptional interference, the lack of cellular or viral activators or the presence of host repressors. Transcriptional repression that induces viral latency is commonly associated with the epigenetic silencing of viral promoters [15] (Figure 1). Here, we briefly present the currently known epigenetic mechanisms that are responsible for the establishment and persistence of episomal and proviral latency.

Chromatin regulation during episomal latency

An extensively studied example for episomal latency is established by the herpes virus family, comprising herpes simplex virus (HSV1). The epigenetic regulation of latent HSV1 gene expression relies on posttranslational histone modifications but not on DNA methylation [16–18]. Establishment of latency is associated with the recruitment of several proteins, including HDACs and polycomb-repressive complex 2 (PRC2) components, to lytic gene promoters [16,18]. Upon viral reactivation, HCF-1, a component of the SET1 and MLL1 HMT complexes, recruits LSD1 and induces H3K4 trimethylation and transcriptional activation of the HSV promoter [19–21].

Chromatin regulation during proviral latency

Proviral latency is established by the family of retroviruses, which includes HIV-1. Upon reverse

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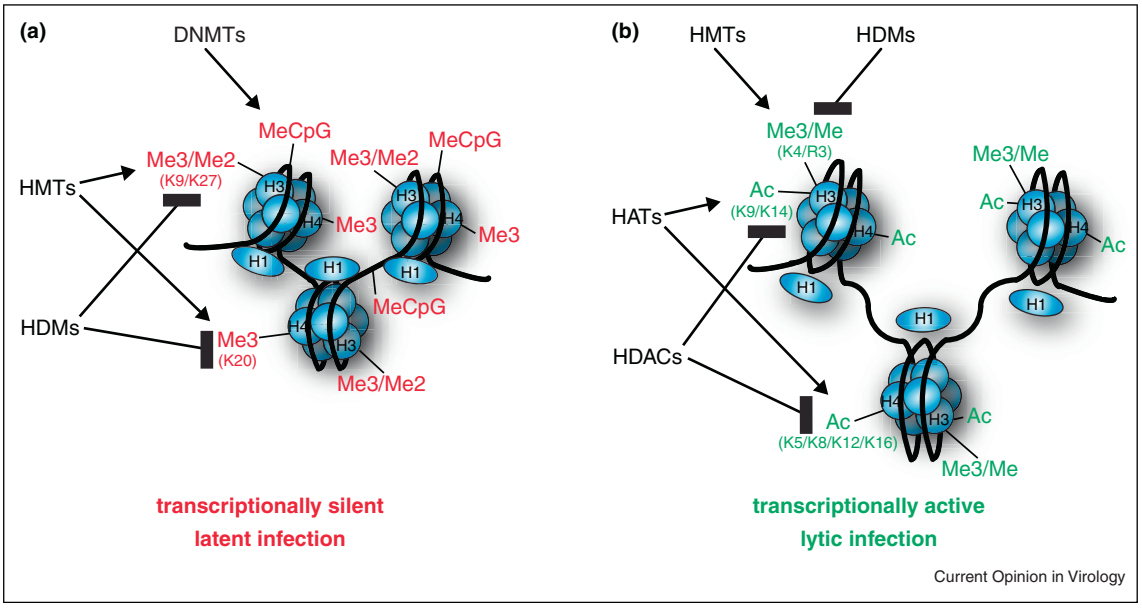
Figure 1



Chromatin structure and viral latency. **(a)** Schematic representation of transcription-repressive heterochromatin at lytic viral promoters, supporting the establishment of viral latency: the posttranslational dimethylation (Me2) or trimethylation (Me3) of histone H3 lysine residues 27 and 9 (H3K27/H3K9) and histone H4 lysine residue 20 (H4K20) as well as the direct methylation of CpG islands (MeCpG) within gene promoters are predominantly associated with transcriptional silencing. Host cellular histone methyltransferases (HMTs) and histone demethylases (HDMs) are responsible for the methylation and demethylation of histone tails, respectively, while DNA methyltransferases (DNMTs) catalyze the methylation of CpG islands. **(b)** Schematic view of actively transcribed euchromatin: the trimethylation (Me3) of histone H3 lysine residue 4 (H3K4) and the monomethylation (Me) of arginine residue 3 (H3R3) as well as the acetylation (Ac) of histone H3 lysine residues 9 and 14 (H3K9, H3K14) and histone H4 lysine residues 5, 8, 12 and 16 (H4K5, H4K8, H4K12, H4K16) are predominantly associated with active transcription. Besides HMTs and HDMs, histone acetyltransferases (HATs) and histone deacetylases (HDACs) are involved in the setting and erasing of these chromatin marks, respectively. Also a decreased binding of the linker histone H1 is involved in chromatin remodeling.

transcription, the retroviral genome is integrated into the host cellular genome, where it either resides transcriptionally active or dormant, leading to virion production or latency, respectively. Epigenetic silencing of viral transcription constitutes the major mechanism involved in both establishing and maintaining latency and, in contrast to episomal latency, relies on both DNA methylation and histone modifications [5,22] (Figure 1). In microglial cells, the viral reservoirs in the CNS, we have shown that the cellular proteins CTIP2 and LSD1 synergistically repress HIV-1 transcription [23,24]. Indeed, CTIP2 recruits HDAC1, HDAC2 and SUV39h1, leading to H3K9 deacetylation and trimethylation, respectively [24]. Surprisingly, LSD1 recruits hSET1 and WDR5, two members of the human complex proteins associated with Set1 (hCOMPASS), to trimethylate H3K4 [23]. Association of both H3K4me3 and H3K9me3 epigenetic marks with LSD1 recruitment may thus constitute a new level of eukaryotic gene regulation. Such a gene repression linked to H3K4me3 has been proposed to prevent the expression of cryptic promoters [25,26]. This is strengthened by the findings that HIV-1 preferentially integrates into active genes and therefore could be considered as a cryptic gene. Interestingly and as described for HSV, a PRC2 complex is involved in epigenetic silencing of HIV-1 by trimethylation of H3K27 [27].

Non-coding RNAs in chromatin regulation

During the last years, an increasing number of ncRNAs has emerged being potently involved in chromatin structural dynamics, acting as recruiting factors, scaffolds or decoys for epigenetic chromatin-modifying enzymes [28]. While certain miRNAs recruit epigenetic regulators via sequence-specific interactions with their target genes, the regulatory mechanisms utilized by lncRNAs are classified into cis and trans (Figure 2) [28]. Here we focus on trans-acting host lncRNAs, viral lncRNAs including long antisense transcripts and viral miRNAs.

Trans-acting long non-coding RNAs

In contrast to miRNAs, lncRNAs predominantly exert their function via intramolecular secondary structure formation, enabling them to specifically bind cellular interaction partners. They can be classified according to their locus and orientation with respect to the surrounding protein-coding genes.

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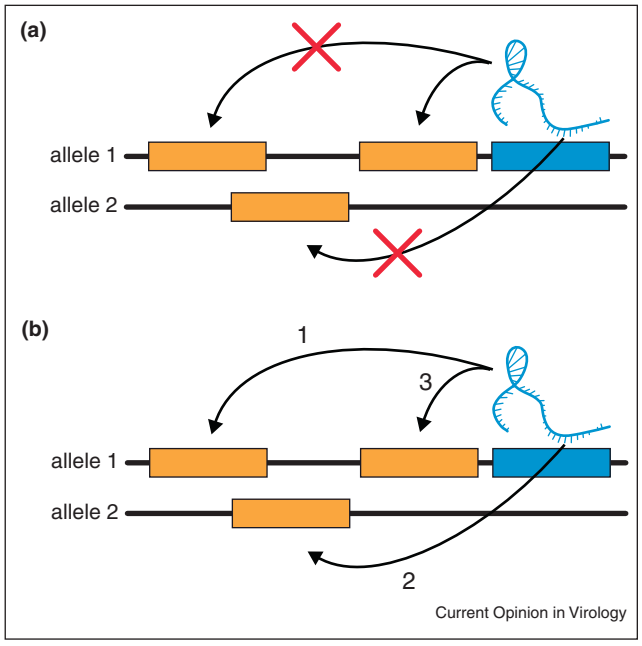
Figure 2



Cis-regulation versus trans-regulation. **(a)** In a cis-regulatory mechanism the expression of the ncRNA is directly correlated with the expression of a neighboring gene on the same allele (adapted from Guttman and Rinn [28]). **(b)** Trans-regulation is believed to be a more general mechanism, which can affect the expression of distant genes (1) or genes located on the other allele (2). Also the expression of neighboring genes can be affected (3), but such an effect here is indirect and involves mediating factors. Thus, apart from cis-regulatory host lncRNAs affecting chromatin structure directly at the site of retroviral integration and apart from viral lncRNAs, lncRNA candidates affecting chromatin structure during episomal and proviral latency are likely to act in trans.

A recently discovered prominent long intergenic (li)ncRNA is the HOX antisense intergenic RNA (HOTAIR), which is transcribed in antisense orientation from the HOXC locus [29]. HOTAIR interacts with PRC2 and mediates PRC2-catalyzed H3K27 methylation at the HOXD locus in trans. HOTAIR is constitutively overexpressed in breast cancer cells, thereby inducing a re-targeting of PRC2, leading to genome-wide altered gene expression [30•]. RNA immunoprecipitation-sequencing (RIP-seq) studies have revealed more than 9000 PRC2-interacting RNAs, including promoter-associated transcripts and lincRNAs transcribed from all over the genome, advancing the regulation of PRC2-mediated chromatin-remodeling to a large variety of ncRNAs [31••]. More recent studies have shown HOTAIR to act as a scaffold between PRC2 and the HDM LSD1, which is believed to coordinate H3K27 methylation and H3K4 demethylation to silence target genes [32••] (Figure 3a). Genome-wide studies have proven about 20% of all cellular lincRNAs to be associated with PRC2, while about 10% showed an interaction with CoREST, a compound of LSD1-containing repressor complexes [33]. Remarkably, 40% of the CoREST-associated lincRNAs also associate with PRC2, indicating that in addition to HOTAIR, many lincRNAs may act as scaffold for chromatin-modifying activities. 74 lincRNAs physically interact with various chromatin-modifying enzymes, among them the HDMs Jarid1b and Jarid1c, HDAC1, PRC2, the HMTs Eset, Setd8 and Suv39h1 as well as the HAT TIP60/P400 [34••], many of which have been previously shown to be involved in HIV-1 silencing [5].

Apart from these host cellular lncRNA examples there is also a variety of viral lncRNAs, which are particularly expressed either during the latent or the lytic phase of infection. Among the latter ones, the Kaposi's sarcoma-associated herpesvirus (KSHV) encoded polyadenylated nuclear (PAN) RNA mediates infectious virion production [35]. It associates with the HDMs JMJD3 and UTX and with the HMT MLL2 to target these activities to the KHSV promoter, leading to increased H3K27 demethylation and H3K4 methylation and thus to transcription activation [36•] (Figure 3b). During HSV latency, only the latency-associated transcript (LAT) is expressed at high levels and promotes heterochromatin formation on lytic promoters in a mouse model [37]. However, this potential function of LAT appears to depend on the animal model used [16] and will need to be further investigated.

Besides the epigenetic modification of core histones also the competitive displacement of linker histones has been implicated in chromatin structural dynamics [38] (Figure 1). In recent studies we have identified the essential 7SK ncRNA as decoy for the histone H1 antagonist HMGA1 [39•,40] (Figure 3c). Apart from its function as a repressor of the positive transcription elongation factor b (P-TEFb) [41], 7SK RNA regulates the expression of more than 1500 HMGA1 target genes. Since P-TEFb activity is also critical for efficient HIV-1 genome expression [42], the recent identification of 7SK complexes containing both HMGA1 and P-TEFb directly links chromatin-remodeling and transcription elongation control during HIV latency [43].

Long antisense transcripts and cis regulation

Unlike other lncRNAs, long antisense transcripts usually regulate their target genes by recruiting epigenetic effectors via a sequence-specific interaction with their complementary nascent sense mRNA in cis [13] (Figure 3d). For instance, the antisense ncRNA in the INK4 locus (ANRIL) overlaps with the locus, which encodes for the cyclin-dependent kinase inhibitors p15INK4b and p16INK4a and the p53 regulator ARF. ANRIL affects the expression of its target genes by two different mechanisms: it mediates p15INK4b repression by PRC2-mediated heterochromatin formation and DNA methylation [44••,45] and it silences p16INK4a expression via

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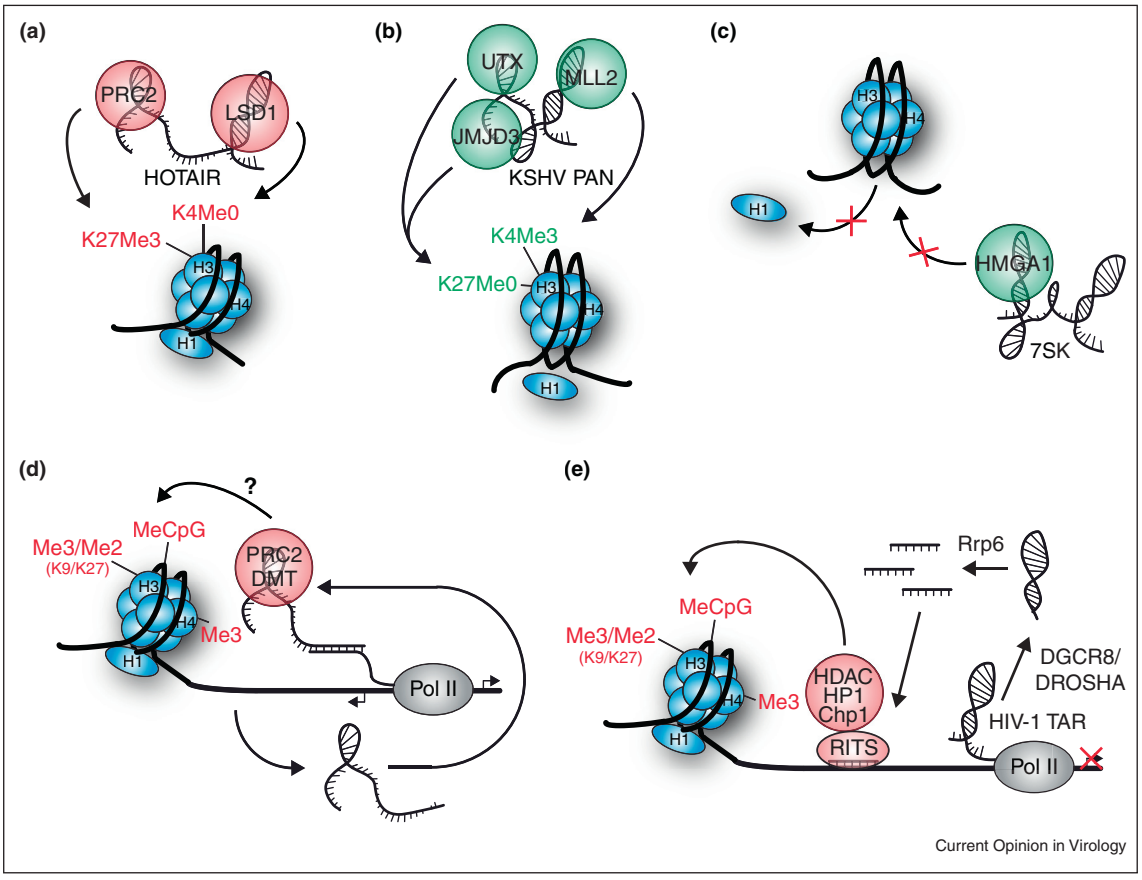
Figure 3



Non-coding RNAs during chromatin regulation. **(a)** The lincRNA HOTAIR acts as a scaffold for the HMT PRC2 and the HDM LSD1 in order to silence gene expression by the coordination of H3K27 trimethylation (H3K27Me3) and demethylation of H3K4 (H3K4Me0). **(b)** The KHSV-expressed PAN RNA activates viral gene expression by recruiting the HDMs UTX and JMJD3 and the HMT MLL2, resulting in H3K27 demethylation (H3K27Me0) and H3K4 trimethylation (H3K4Me3), respectively. **(c)** Cellular 7SK RNA acts as decoy for the histone H1 antagonist and chromatin master regulator HMGA1. **(d)** Long antisense transcripts, such as ANRIL, are involved in the recruitment of chromatin-modifying enzymes (PRC2, DNMTs?) to gene promoters by basepairing with their complementary sequence within the nascent mRNA, resulting in gene expression silencing. **(e)** HIV-1 TAR is processed by DGCR8, DROSHA and Rrp6 to miRNAs, which bind to their promoter proximal target sites and are believed to recruit RITS-associated chromatin-modifiers (HDACs, HP1, Chp1), which subsequently mediate HIV-1 silencing.

the recruitment of the HMT EZH2 and PRC1-compound CBX7 [46•].

Recent studies have demonstrated antisense transcription to occur also from the integrated HIV-1 genome [47]. A long antisense RNA transcribed from the U3 region of the 3′ LTR has been detected in infected cells [48••]. Though the expression of this ncRNA affects gene expression in a repressive manner, until now cellular interaction partners remain unidentified, leaving the mechanism of gene repression elusive.

Also the target of the antisense transcript itself, the nascent viral mRNA may directly serve as a cis-regulatory element. We have recently shown the HIV-1 transactivating response (TAR) structure to bind the chromatin regulator HMGA1, resulting in diminished HIV gene expression [49]. In addition to the competitive inhibition of the interaction with Tat-activated P-TEFb, this HMGA1/TAR complex might also play important roles during chromatin remodeling at the viral promoter.

Viral microRNAs

The best characterized pathway of miRNA-mediated gene repression is posttranscriptional silencing via the RNA-induced silencing complex (RISC) [50]. Apart from that, more recent studies have revealed that such short transcripts can also target epigenetic heterochromatin formation [51]. By guiding the RNA-induced transcriptional silencing (RITS) complex to their complementary target sites, these RNAs recruit RITS-associating epigenetic silencers, such as the HMT Clr4 [52], the DMT Dnmt3a [53] or HDAC1 [54]. However, even though both viral and host cellular miRNAs have been shown to affect

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viral gene expression at the posttranscriptional level [55], there is only little known about miRNAs involved in the epigenetic regulation of viral target promoters.

In the case of HIV, the TAR element is a source for such miRNA species. It is recognized and cleaved by Dicer, producing miRNAs, which silence viral gene expression by facilitating the recruitment of HDAC1 to their promoter proximal target sites [54]. More recent studies have shown microprocessor to mediate premature termination of transcription upon TAR synthesis by recruiting the termination factors Setx and Xm2. The TAR element is subsequently cleaved by Drosha and processed by the exonuclease Rrp6 into miRNAs, which are believed to direct epigenetic silencing enzymes via the RITS pathway [56•] (Figure 3e).

Conclusions

Viral latency constitutes a serious obstacle for the cure of viral infection. The antiretroviral treatment (ART) of HIV infected patients for instance is able to target actively virus-producing cells, but fails to attack latently infected cells, necessitating lifelong ART. A better comprehension of the mechanisms underlying establishment and persistence of HIV-1 latency will help to design therapies based on reactivation of reservoirs, which combined with an improved highly active ART could lead to a functional cure [57–60]. A repressive chromatin structure at the viral promoter has emerged as a critical prerequisite for latency establishment [15], making host cellular epigenetic regulators important players during the silencing of viral gene expression.

Though in most cases a wide range of cell types can be infected, often certain cell types are more prone to latency than others, such as CD34+ mononuclear hematopoietic progenitor cells in the case of cytomegalovirus (CMV) [61] or CD4+ T and monocyte–macrophage cells during HIV-1 infection [60,62], supporting the idea of cell-specific factors being major players during latency establishment.

Non-coding RNAs are gaining increasing attention as scaffolds and recruiting factors for chromatin regulators and thus may establish a novel, cell type specific layer during epigenetic regulation. Most of the known complexes involved in the establishment of heterochromatin-associated viral latency can be globally or specifically targeted by host cellular and/or viral ncRNAs to gene promoters. Notably, the expression of ncRNAs differs by far more than the expression of protein coding genes when comparing different cell types and tissues, making them promising candidate players during the chromatin-mediated establishment of viral latency [63,64••].

Deeper insights into ncRNA species specifically expressed in those cell types, which are prone to viral latency, will be required in order to identify RNAs participating in the epigenetic repression of viral genes. Such ncRNAs could represent novel therapeutic targets in order to eradicate latent viral reservoirs, which could in turn support ART to cure retroviral infection. Especially systems approaches applying recent powerful technologies such as next generation sequencing — as pioneered by Peng *et al.* [65] and Chang *et al.* [66•] — RNA-seq, RIP-Seq or 5′mC-Seq will substantially improve the detection of regulatory ncRNA networks during viral infection to advance our current knowledge about the establishment of viral latency and host-pathogen interactions in general [67].

Conflict of interest statement

The authors declare to have no competing interests.

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